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ABSTRACT

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As indicated by most parameters employed, rats tend to adapt after 12 weeks of constant exposure to an environment composed essentially of oxygen at 5 psia. The use of injected acetate-1-C^{14} to study the rate of conversion of acetate to CO_2 has been justified by evidence which showed that the size of the acetate pool in the liver was not influenced by the test environment.

Further, it has been shown that the rate of conversion of C^{14}-acetate to $C^{14}O_2$ was slowed but that the steady state rate of total CO_2 expiration was not altered in the experimental animals which were exposed to the high oxygen-low pressure environment for 4 weeks. The metabolic implications of these data are discussed.

The amount of ascorbic acid found in the adrenal glands was considerably reduced in animals exposed for 4 weeks to the test environment. However, evidence suggested no apparent change in adrenal function in response to stress as measured by adrenal weight and cortical width measurements.

The eyes of animals exposed for 4 weeks to the high oxygen-low pressure environment contained 33% less lipid than the

control animals. The decrease appeared to be a general one involving all classes of lipids. Additionally, the eyes weighed less and the difference could not be accounted for simply on the basis of lipid.

Author

This report includes the abstract of a paper entitled "Adaptation of Rats to a High Oxygen-Low Pressure Environment" by Jordan, Allred and Bond which is to be presented at the 1966 Fall Meeting of the American Physiological Society. A general discussion relating the accumulative evidence to date is included also.

I. INTRODUCTION

Our most recent Status Reports have presented evidence to suggest that after prolonged exposure to an environment essentially made up of oxygen at 5 psia, animals tend to adapt to the environment. However, to complete the evidence preliminary to preparation of manuscripts, a number of other aspects of the total 12 weeks exposure period and its effect upon the animals required clarification.

The relationship of stress to adrenal function in general has been well established. This report includes evidence showing that the ascorbic acid content of the adrenal glands was reduced in the experimental animals after 2 and 4 weeks of exposure but that there was no evidence of a change in the size of the adrenal cortex. Additionally, since the interpretation of our data on the turnover rates of metabolic pools is based on the assumption that the acetate pool size in the experimental animals is essentially comparable to that of the control animals, this fact had to be established. Based upon this assumption, the decision was made to inject the animals on a per kilogram of body weight basis. This report includes evidence to substantiate the validity of that assumption.

We have been concerned previously with the possibility of creating artifacts in our isotope experiments by the removal of animals from the experimental environment for a brief period preceding their sacrifice. Evidence has been obtained that no artifacts were created since animals removed from this environment and maintained in metabolic chambers for 8 hours incorporated C^{14}-acetate into liver lipids to the same extent as experimental animals maintained in the test environment. Additional data concerning heart and carcass lipids is also presented.

Because of the importance of the eye to an astronaut and the susceptibility of this tissue to oxygen toxicity, eye lipids were investigated and found to be reduced to 67% of the control values in animals exposed to the test environment for a period of 4 weeks. Additionally, the weight of the experimental animal eyes was somewhat reduced. To date no evidence has been gathered to indicate preferential destruction of one type of lipid over another. Further, no turnover rate data are yet available on eye lipids so that no conclusion can be made with respect to the relationship of biosynthesis to catabolism in eye lipids.

Finally, this report includes a preprint of an abstract of an article entitled, "Metabolic Adaptation of Rats to a High Oxygen Low Pressure Environment" by Jordan, Allred, and Bond, which will be presented at the fall meeting of the American Physiological Society to be held at Houston, Texas. During the period of this report, a paper entitled "Effect of Discontinuous Exposure of Rats to a High Oxygen-Low Pressure Environment" by Jordan, Allred, Cahill and Clark was published in Aerospace Medicine, 37:368, 1966.

II. EXPERIMENTAL

During the six months period covered by this report, tissue composition and metabolic activity as measured by $C^{14}O_2$ expiration were studied using animals that had been exposed to an environment essentially made up of oxygen at 5 psia. Animals were exposed to the test environment for periods of two, four or twelve weeks, and were raised under the conditions described by Kollias and Jordan (1).

A drawing of the complete system (Figure 1) shows the large decompression chamber with its air lock. Two small chambers, each holding eighteen to twenty-one rats can be seen inside the main chamber room. The control console for the small chambers, the readout system and the circulating water bath are shown diagramatically as blocks. The master control console for the large chamber is also observable. Each of the small chambers (Figure 2) is maintained at a relatively constant temperature by circulating water through a jacket surrounding each chamber. The temperature readout system, drinking water system, and altitude control and oxygen delivery system operate as described by Kollias and Jordan (1). Note that the

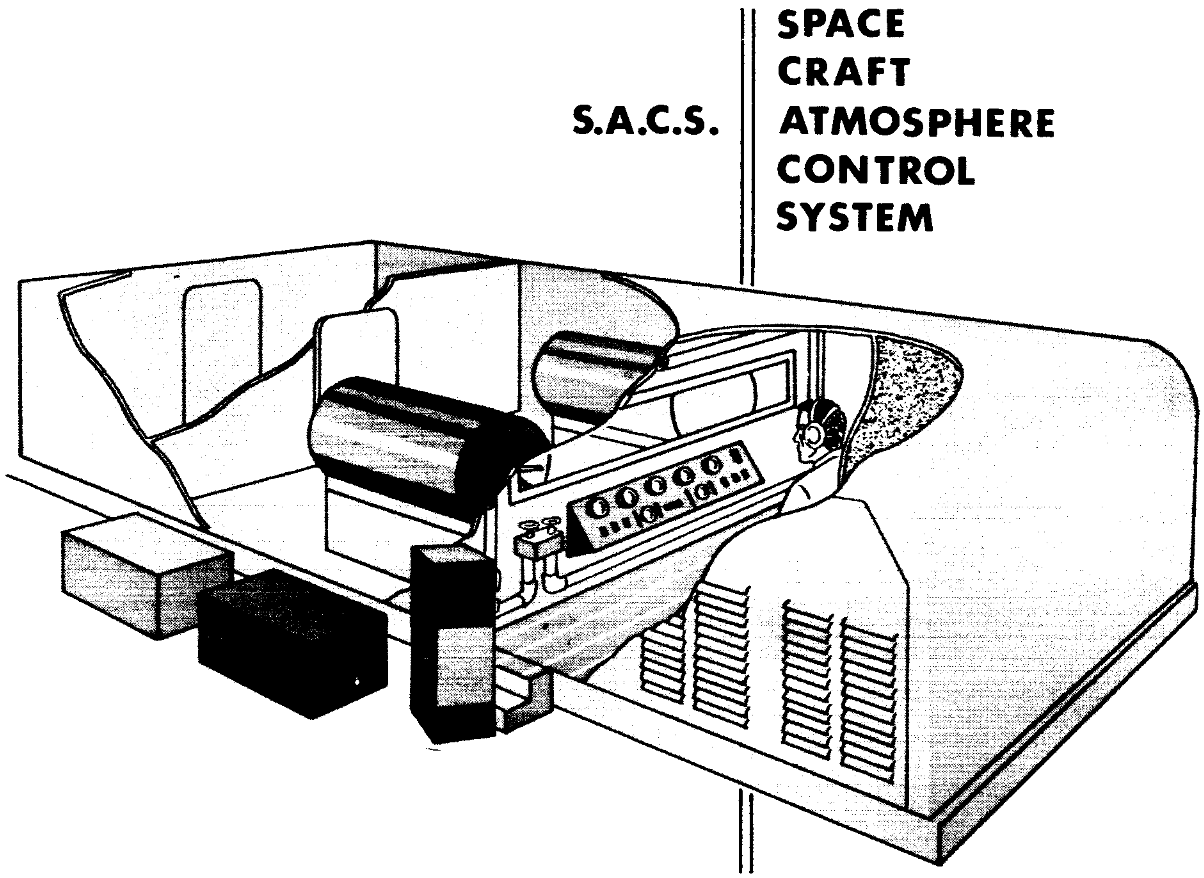


Figure 1. Cut-away Drawing of Space-Craft Atmosphere Control System. One of the small animal chambers houses experimental animals in O_2 at 5 psia; the other contains control animals in air at 1 atmosphere.

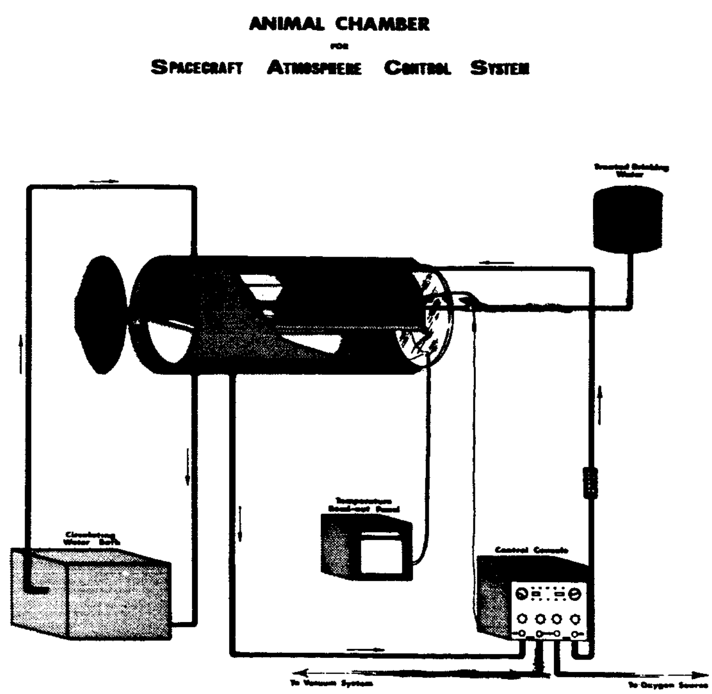


Figure 2. Cut-away Drawing of Animal Chamber Complex.

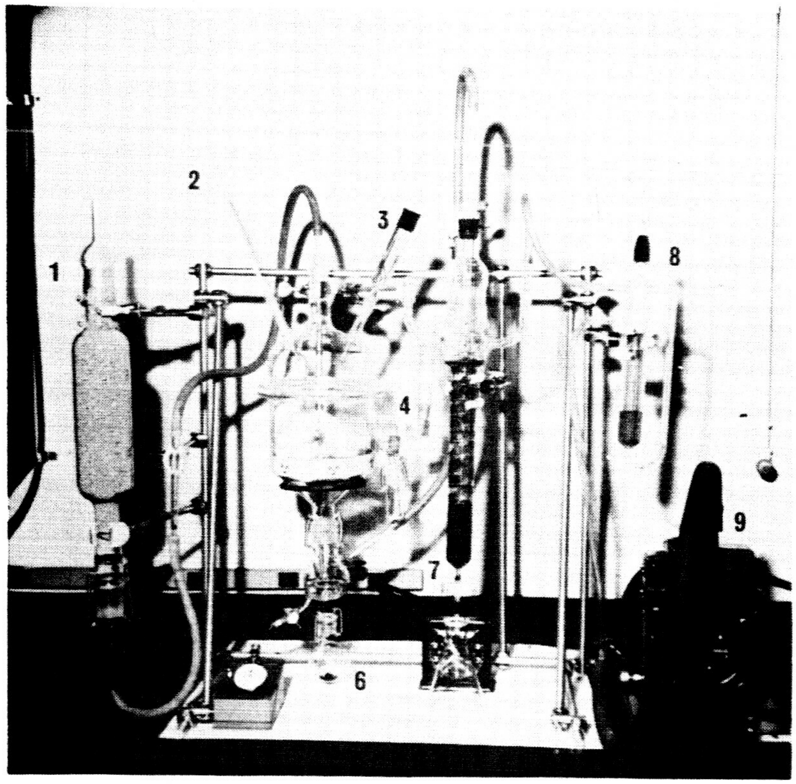
animal activity can be observed through the plexiglass plate at one end of each chamber. The feeder can be observed on the wire gauze walkway.

Food is replenished and drop trays are removed at least twice a week by decompressing the large chamber to 5 psia with a technician inside. The door of the experimental chamber is opened and the chamber serviced. During this period a barometer hooked to the control chamber registers any change in pressure inside the control chamber. It never changes more than 10 mm. Obviously the control chamber is serviced at one atmosphere pressure. The flow rate of air through the control chamber is comparable to the flow rate of oxygen to the experimental chamber.

The water is treated with tylan tartrate at the level of 500 μg per gallon. This is a subclinical, prophylactic level for respiratory problems which tend to be accentuated in the crowded conditions of the chamber. During the past six months, two four-week experiments were essentially aborted because most of the animals in both the control and experimental chambers came down with pneumonia. Consequently no metabolic studies were made using any of these animals. It should be clearly indicated that there is no evidence to suggest that the high oxygen low pressure environment had any particular influence on the susceptibility of these animals to respiratory problems, since in each experiment approximately the same number of animals in the control and experimental chambers possessed symptoms of respiratory disease.

For C^{14}-labeled metabolic experiments, animals were injected with acetate-1-C^{14} (2μc/μmole) at the rate of 700 μc/kg of body weight. In one experiment the level was reduced to 150 μc/kg. To determine the validity of the C^{14} metabolic experiments, the size of the acetate pool was determined in the liver and similar work on the carcass as a whole is in progress. Since measurement of metabolic activity with respect to acetate is determined by expiration of $C^{14}O_2$ as well as incorporation of radiosubstrate into metabolic pools, paired groups of controls and experimental animals were injected with $NaHC^{14}O_3$ (23 μc/mM) at the rate of 25 μc/kg of body weight. Additionally the total expiration of CO_2 over an eight hour period was measured. The specific experimental procedures used in these experiments are covered in the appropriate sections of this report. The aggregate of data from the CO_2 expiration experiments was used to ascertain whether there was an impediment in the transport of gases across the lung. An alternate method using CO is currently in progress.

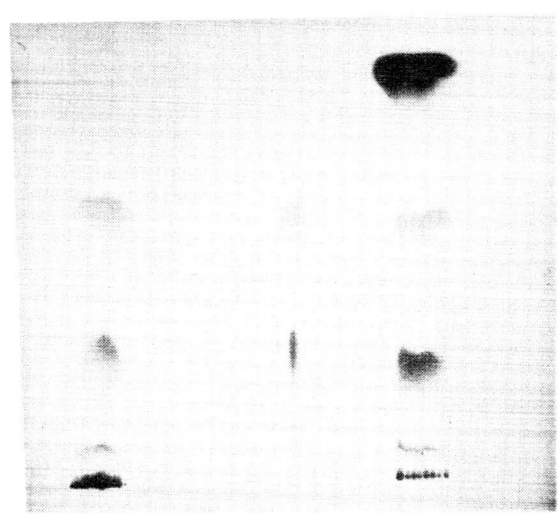
To trap the CO_2 expired from animals injected either with acetate-1-C^{14} or $NaHC^{14}O_3$, animals were injected and placed in metabolic chambers (Figure 3) for eight hours. Samples were collected continuously by bubbling the expired air through 0.2M NaOH. Fresh NaOH was replaced in the reservoir periodically (each ten minutes for the first hour post injection, each twenty minutes during the second hour, and each thirty minutes for the remainder of the eight hour period). The total CO_2 expired during this period was measured by titration while the measurement of radioactivity was obtained by precipitating the CO_2 as $BaCO_3$ which was suspended in a dioxane-POPOP-water counting solution by ultrasonication and was counted in the Tri-Carb liquid scintillation spectrophotometer. The details of the technique are essentially those outlined in the October 1964 Status Report.



1. Air Intake CO_2 Trap
2. Thermometer
3. Drinking Water
4. Feed
5. Urine Trap
6. Solid Excretion Trap
7. Expired CO_2 Trap
8. Vacuum Regulator
9. Vacuum Pump

Figure 3. Metabolic Chamber for CO_2 Expiration Studies.

Expt'l Std Control



Unknown

Saturates

Mono- & Dienes

Trienes

Origin Tetraenes

Figure 4. Thin-layer Chromatogram of Methyl Esters of the Carcass Fatty Acids. (Origin at bottom; sample from control animal on right, experimental animal on left. Standard contains methyl esters of saturated and mono-unsaturated fatty acids. See text for details.)

To check the validity of the data obtained from experimental animals returned to one atmosphere pressure, a challenge experiment was initiated utilizing paired animals which were injected at 5 psia with radioacetate-1-C^{14} and replaced in the metabolic chamber to metabolize over an eight hour period. By comparing the incorporation of C^{14}-acetate into liver lipids in the animals allowed to metabolize in the test environment and those handled at atmosphere pressure (Section VI), it should be possible to observe any appreciable change in the metabolism of this substrate by experimental animals returned to one atmosphere.

Fractionation of heart lipids was accomplished with the aid of thin-layer chromatography as outlined in our April 1965 Status Report. Determination of turnover rates of these lipids was accomplished as reported in our October 1965 Status Report. Fractionation of the saponifiable carcass lipids was accomplished by esterifying the acids with BF_3-methanol according to the procedure of Metcalf and Schmitz (2) and separation of the methyl esters of the fatty acids on silver nitrate impregnated silica gel thin layer plates. The plates were developed in n-hexane:diethylether (90:10). The lipids were visualized either with the aid of a flourescine dye or charred after spraying with 10% sulfuric acid solution (Figure 4).

III. ADRENAL FUNCTION

A. Introduction

A very important question underlying research of this type and its ultimate application to man is whether the experimental animals are undergoing the very general and vaguely defined condition known as "stress." This condition has long been associated with adrenal function such that the animal's endocrine system will recognize the situation and cause increased amounts of ACTH to be secreted. Theoretically, the ACTH promotes increased production of adrenal corticoids which in turn bring about internal adjustments to allow the individual to cope with the stressful stimuli.

In order to pursue this possibility, we chose some relatively simple and fairly gross parameters for study: overall adrenal weight and cortical width as a measure of ACTH stimulation on growth of the adrenal cortex and depletion of adrenal ascorbic acid as an index to ACTH stimulation of adrenal steroidogenesis.

B. Methods

All animals were sacrificed at 1 atmosphere pressure except in experiment 3 in which the experimental animals were sacrificed at 5 psia. Both adrenal glands were removed immediately after sacrifice. The glands were immediately cleared of fat and weighed. In experiment 1, both glands were placed in Bouin's fixative at room temperature in preparation for histological study. In experiments 2 and 3, one gland of each pair was treated as before while the other gland was homogenized in cold 2.5% metaphosphoric acid for ascorbic acid determination. In these cases, cleaning and weighing was done in the cold room at 34°F.

(a) Ascorbic Acid Determination: The Fresh gland was homogenized with a small hand unit containing 5 ml. of the 2.5% metaphosphoric acid and was filtered through Munktells No. 00 paper. An additional 5 ml. of acid rinsed the homogenizer and was added to the homogenate. Determination of ascorbic acid in the filtrate was accomplished within a few hours with the solutions being maintained in an ice bath throughout the procedure. The quantitative basis for this determination is the ability of ascorbic acid to reduce 2,6-dichlorophenol indophenol measured spectrophotometrically at 515 mμ (Bausch & Lamb Spectronic-20) according to the procedure of Mindlin and Butler (3).

(b) Histological Studies: Excess picric acid was removed from the fixed tissue by soaking in 70% ethanol containing 10% NH_4OH. Dehydration was accomplished by soaking in 70%, 80%, 95% ethanol and two changes of absolute alcohol for about 3/4 hours each. The clearing agent was toluene. The glands were imbedded in small individual tissue-mat blocks.

The blocks were sectioned on the rotary microtome to a thickness of 10 micra. Sections used were those considered closest to the center of the gland or the area of the most extensive medulla. Mayer's albumen adhesive was applied to each slide.

The sections were stained with Harris' Hematoxylin (5 minutes) and counterstained with eosin (3-5 minutes). The finished slides were observed microscopically for total cortical width and for the combined width of the zona fasciculata and zona reticularis. Each cortex was measured at ten places and the average used in computing the width.

C. Results and Discussion

The most obvious and consistent result was the significant depletion of adrenal ascorbic acid level in the experimental animals after 2 or 4 weeks of exposure to the high oxygen-low pressure environment. Although involving relatively few animals

TABLE 1

ADRENAL FUNCTION AS INFLUENCED BY HIGH OXYGEN-

LOW PRESSURE ENVIRONMENT

	Expt. 1		Expt. 2		Expt. 3	
	Control	Exptl	Control	Exptl	Control	Exptl
n=	16	17	3	3	7	7
Length of Expt.	4 weeks		2 weeks		4 weeks	
Adrenal wt. (mg% ± std dev)	11.5 ±1.6	12.7 ±2.6	17.5 ±0.7	20.0 ±3.3	14.4 ±1.2	13.4 ±1.5
Total Cortical Width (mm ± std dev)	0.879 ±0.053	0.854 ±0.060	0.916 ±0.032	0.918 ±0.115	0.910 ±0.082	0.862 ±0.052
F&R Width (mm ± std dev)	0.782 ±0.018	0.771 ±0.062	0.814 ±0.032	0.832 ±0.118	0.827 ±0.083	0.770 ±0.055
Ascorbic Acid (mg% ± std dev)			392 ±35	257* ±10	491 ±35	312* ±30

*Significantly less than the control animals ($P < 0.01$)

(3 per group after 2 weeks of exposure and 7 per group after 4 weeks), the data appear reliable since there was no overlap of values between experimental and control animals. Measurements of adrenal weight, total cortical width, as well as fasciculata and reticularis widths did not yield statistically significant differences between the two groups of animals in any of the three experiments.

If the experimental animals live in a stressful environment, an increase in ACTH secretion leading to hypertrophy of the adrenal cortex and a decreased level of adrenal ascorbic acid would be anticipated. Under such conditions, it is thought that the decrease in ascorbic acid concentration is a result accompanying the primary response of steroid biosynthesis. Under these conditions, hypertrophy of the adrenal cortex is also expected especially in the zona fasciculata and reticularis layers with a concomitant increase in adrenal weight. Clearly, neither of the latter was demonstrated.

It does not appear, therefore, that the experimental animals secreted appreciably more ACTH. It is possible that the indicators chosen were simply too insensitive to reflect very subtle changes in ACTH secretion, and perhaps techniques such as measurement of adrenal steroid output, and ACTH levels in the blood or in the pituitary gland should be attempted.

There is, however, an alternative explanation which in fact seems more plausible. Perhaps the depletion of adrenal ascorbic acid is not related to ACTH secretation at all, but may be a general oxidative response previously observed in other cofactors (e.g. coenzyme A) which may be a reflection of the general hyperoxic state in the experimental animals.*

It is also worth noting that adrenal ascorbic acid values are similar whether the animals are sacrificed at altitude or at 1 atmosphere of pressure, thus indicating that the differences noted are not due to the descent from altitude or the time required for recompression.

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*See blood gas data, coenzyme A concentration and glucose-6-phosphate dehydrogenase levels, etc., reported in our October, 1964, and April, 1965, Status Reports.

IV. ACETATE POOL IN LIVER

A. Introduction

The use of radioactive acetate in previous experiments and interpretation of the data have depended upon the assumption that the acetate pool size is the same in experimental and control animals. The amount of acetate in liver from control and experimental animals exposed for 4 weeks to the high oxygen environment has now been measured using an isotope dilution method and was found to be essentially the same.

B. Methods

After sacrifice, livers were removed and frozen. A portion of the frozen liver, usually 10 to 12 gms., was weighed and homogenized in 9 volumes of 10% KOH solution to which had been added a known amount of sodium acetate-1-C^{14}. The homogenate was refluxed for 30 minutes, made acidic with concentrated HCl and steam distilled. The steam distillate was brought to pH 11-12 with KOH and evaporated to dryness using a rotary evaporator. To the dried material, 0.4 ml of concentrated HCl was added, followed by 0.4 ml of propylene oxide to react with the excess HCl. 2.0 ml of dioxane was added as solvent. Acetic acid was separated and quantitated using gas liquid chromatography.

Radioactivity was determined using a Tri Carb liquid scintillation counter with a dioxane-ethyl alcohol counting solution. From these data, specific activity of the acetate was calculated. Knowing the amount of radioactivity added and the specific activity of recovered acetate, the amount of acetate per gram of liver could be calculated.

C. Results

The quantity of acetate, in terms of micromoles per gram of liver, is shown in Table 2. Although considerable variation among animals was found, no evidence was obtained indicating a difference in liver acetate pool size. Because of the individual variation, acetate pool size will be determined on more animals at a later date. The one high value for an experimental animal (Table 2) was checked and the repetitive determination yielded approximately the same result. The reliability of the method has been established by using known amounts of sodium acetate and found to be accurate within $\pm$ 5%.

The absolute amount of acetate in liver was also of considerable interest since the isotopic tracer technique assumes that the amount of isotope injected is small compared to the pool size in the animal. In our experiments, approximately

TABLE 2

LIVER ACETATE CONTENT OF CONTROL AND EXPERIMENTAL ANIMALS

	μM/gm of liver	Mean	± Std. dev.
Control	24.3, 15.0, 23.8	21.0	± 4.3
Experimental	21.7, 19.7, 42.9	28.1	± 10.5

11 micromoles of acetate were injected into the intact animal per gram of liver. Thus, if the injected acetate is carried directly to the liver rather than being distributed throughout the body or if liver acetate is a significant portion of the total body acetate pool, the injected acetate cannot be considered "tracer" amounts. It should be noted, however, that the fact that the amount of acetate injected may be higher than "tracer" amounts does not alter our previous interpretation of radio-active turnover-rate data, since the total amount of acetate was apparently the same in experimental and control rats. These data do indicate that smaller amounts of radio-active acetate with a higher specific activity should be used in future experiments. Measurement of acetate in the remainder of the carcass is now in progress.

The form in which acetate occurs in liver is of interest because of our previous observation that coenzyme A is reduced in animals subjected to the experimental environment for four weeks. Preliminary observations in our laboratory indicate that almost all of the liver acetate is protein bound rather than occurring as acetyl CoA. Acetyl CoA content in rat liver has been reported to be approximately 20 mμmoles per gram of tissue (4). Coenzyme A, by our measurements (5) is on the order of 400 mμmoles per gram of liver in control rats. Thus, the lack of difference in acetate pool size between experimental and control animals is not inconsistent with the previous observation that the CoA level is reduced in the experimental rat liver.

V. LIPIDS

A. Eye Lipids

The presence of lipids in various parts of the eye has been amply demonstrated (6), and the influence of eye diseases on eye lipids has been reported (7). The importance of lipids in the eye is not completely understood but its involvement in the solubility of visual pigments and its relationship to visual reception have been investigated (8, 9).

Studies on the effects of 100% oxygen environments on the eye suggest that the cone cells are more resistant to oxygen toxicity than rod cells in the same manner that they tend to resist the effects of iodoacetate and X-irradiation (10-12). Further, the oxidation of some fatty acids and their esters by the crystalline lens of the scorbutic guinea pig has been reported (13). The eye is of particular interest, since along with the skin, it is one tissue that is directly exposed to the high oxygen content of the environmental chambers. One would expect the eye to be a rather sensitive tissue in which to investigate the effects of oxygen toxicity, even under conditions of reduced pressure.

The rat eyes were removed intact from rats as soon as possible after sacrifice and washed in 0.25 M sucrose. The eyes were cleaned of excess muscle tissue, blotted and weighed. The lipids were extracted from the eyes by the chloroform:methanol:water method of Bligh and Dyer (14). The extracts were then placed in pre-weighed, desiccated (24 hours) vials and taken to dryness under nitrogen. The vials were then desiccated an additional 24 hours and re-weighed. The weight of the lipid material was determined by difference.

The data (Table 3) indicate that the experimental animals' eyes were somewhat lighter in weight than the comparable control animals. However, the weight of lipid was boldly different although the distribution of weight between the saponifiable and nonsaponifiable fractions was not different statistically in the experimental animals compared with the control animals according to Student's T Test. No C^{14}-acetate incorporation studies have been made as yet but are planned for the summer of 1966. The eye lipid data to date have been obtained from animals exposed to the high oxygen-low pressure environment for 4 weeks.

TABLE 3

EFFECT OF 4 WEEKS OF EXPOSURE TO HIGH OXYGEN-LOW PRESSURE ENVIRONMENT ON EYE WEIGHT AND EYE LIPIDS

Animal	Eye Wt. (gm)	Wt. of Lipid(%)	Types of Lipids (wt)	
			%Saponifiable	%Nonsaponifiable
Control	0.225	2.62	65	35
Experimental	0.200	1.76	76	24

B. Heart Lipids

Our October, 1965, Status Report included data relative to the rate of incorporation of C^{14}-acetate into heart lipids after both four and 12 weeks of exposure to the test environment. The respective lipids were separated by thin-layer chromatographic techniques as outlined in that report. It was observed that after twelve weeks of exposure there seemed to be little difference in the fractionated heart lipids between experimental and control animals, except for the turnover rate of certain fractions. However, one bold difference was observed. After two hours of metabolizing radioactive acetate, incorporation into

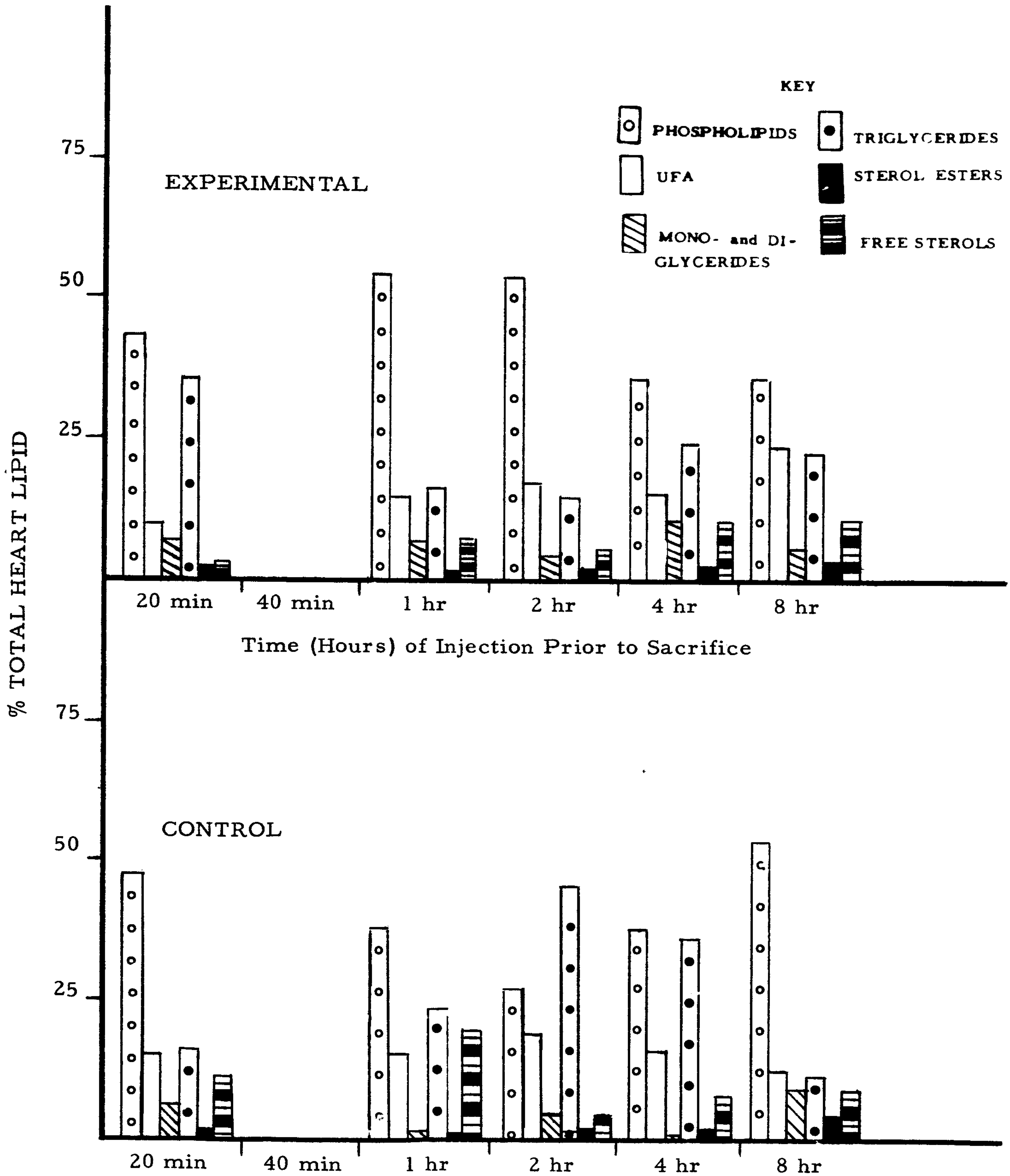

Figure 5. C^{14} Incorporation into Heart Lipids (% of total C^{14} incorporated into Heart Lipids) After 12 weeks of exposure

unesterified fatty acids appeared much greater in the control animals. These particular data appeared out of line and suggested the need for a challenge experiment. Figure 5 shows the entire set of data for the twelve weeks exposed animals including the data from the challenge experiment on the animals allowed to metabclize two hours.

It is apparent when the initial data were obtained some difficulty in separating the unesterified fatty acid fraction from the triglyceride fraction must have occurred. The TLC technique employed places these two fractions next to each other. These data are generally in line and indicate that there was no unusually high incorporation of radioacetate in the unesterified fatty acid fraction. It is of interest however to note that in general there is greater incorporation of C^{14}-acetate into the triglyceride fraction in the control animals. Such a difference was not apparent after only four weeks of exposure to the experimental environment. How these data relate to the increase in heart size is interesting to ponder.

C. <u>Carcass Lipids</u>

With prolonged exposure of animals to the 5 psia-100% oxygen environment, we have already reported a decrease in saponifiable lipids (5). Our last Status Report also showed a difference in the turnover rate of the saponifiable fraction of the carcass lipids. Further, we have reported a difference in the incorporation of C^{14}-acetate into the fatty acids of the liver (15). In that particular experiment the ratio of unsaturate to saturated fatty acids increased in the experimental animals. At the time this was a surprise to us since one might have expected in a hyperoxic state (and these were animals exposed for four weeks to the test environment) that lipid peroxidation would have increased; consequently, the unsaturated fatty acids would be decreased. However, evidence from Meade's Laboratory (19) indicates that animals can synthesize mono-unsaturated fatty acids by direct conversion from the corresponding saturated fatty acids in the presence of NADPH and molecular oxygen.

With these background data we have continued our analyses of carcass lipids by submitting the methyl esters of the saponifiable lipids to separation on thin-layer chromatography. The technique involved the spreading of Silica Gel G on glass plates and spraying with a 25% solution of silver nitrate. The plates were then activated in an oven at 110°C for one hour. Lipids were spotted and developed in n-hexane: diethylether (90:10) in about 40 minutes. If the lipids were to be recovered, they are visualized by staining with the 0.1% solution 2, 7-dichlorofluorescein in aqueous ethanol (1:1) and viewed under ultraviolet light (16). If the amount of radioactivity in the sample was sufficient, the plate was scanned on a Berthold Radioscanner (Dunnschicht Scanner). If the amount of radioactivity was not great enough to use the radioscanner, the separated lipid material was extracted from the absorbant and the indicator by the procedure of Privett, et al (17).

If a photographic record of the plate was to be made, the spots were made visible by spraying with a 10% sulfuric acid solution and heated at 250°C to char the organic components. Figure 4 shows a standard in the middle of the plate which contains the methyl esters of myristic, palmitic and stearic acids as the saturates, and palmitoleic and oleic acids as the unsaturates. The standard spot closest to the solvent front contains only the saturated fatty acid methyl esters while the second spot contains the unsaturated acids. The tri- and tetraenes are not seen in the standard but are observable in the carcass lipid samples as the bottom two spots. Note that the control animals have a very large spot that moves approximately with the solvent front. This spot is almost completely absent from the experimental carcass lipids. The identification of the spot is not yet complete but the spot does not co-chromatograph with short chain fatty acid methyl esters such as methylhexanoate nor with the methyl esters of hydroxy fatty acids such as methyl-12-hydroxy stearate, nor with the methyl esters of branch-chain fatty acids out to C_{21}. It is evident that the molecular weight of the compound(s) must be relatively small and it must be relatively non-polar to move in the solvent system as it does. The significance of this observation must, of course, await identification of the spot. Caution should be employed to prevent misinterpretation of these data in quantitative terms since we have already presented evidence (18) that charring with sulfuric acid and heat is not a satisfactory quantitative technique except within a particular group of compounds. For example, the presence of a single unsaturated bond increases the amount of charring tremendously. Similarly, ring structures and other modifications of structure can grossly influence the degree of charring. Therefore, although it appears that there is considerable amount of this material in the control animal carcass lipids, true quantitation of this material must await further laboratory work.

VI. VALIDATION OF METABOLIC CHAMBER DATA

Early work in this program involving studies of the long term (more than 1 day) turnover rate of metabolic pools was based upon the injection of acetate-1-C^{14} into experimental animals at altitude and the replacement of these animals in the test environment for the duration of the metabolizing period. However, during more recent short term turnover rate studies (8 hours or less) we have used metabolic chambers at 1 atmosphere to aid in the short term collection of $C^{14}O_2$ expiration data. In these experiments the experimental animals have been removed from the test environment, injected with substrate and placed in a glass metabolic chamber with air as the gas phase at 1 atmosphere. We have been somewhat concerned about the validity of data obtained from such experimental animals, not knowing the short term effect of changes in the gaseous environment on metabolism.

It was, therefore, determined to inject two sets of animals in a paired experiment. One set of animals was replaced in the test environment and the other set was placed in metabolic chambers at 1 atmosphere. After metabolizing for 8 hours, the incorporation of the C^{14}-acetate into liver lipids was used as the indicator. Both sets of animals were injected at the rate of 150 μc per kilogram of body weight. The average total incorporation into liver lipids was almost identical in the two groups (Table 4). We conclude from these data that with respect to C^{14}-acetate metabolism, the data collected to date using metabolic chambers or replacing animals in the test environment are both valid and comparable sets of data.

TABLE 4

EFFECT OF CHAMBER ENVIRONMENT ON SHORT TERM (8 HR) METABOLISM

(Animals were injected with acetate-1-C^{14} and allowed to metabolize for 8 hrs as indicated)

Experimental Animal Environment	No. of Animals	Incorporation of C^{14}-acetate into Liver Lipids (dpm)
Replaced in High O_2-Low Pressure Chamber	3	461,817
Placed in Metabolic Chambers at 1 Atm.	3	485,950

VII. RESPIRATORY CO_2

Determination of radioactivity in expired CO_2 from acetate-1-C^{14} was again measured in control animals and animals exposed to the experimental environment for four weeks. The procedure, which has been described in detail previously (see Status Report October, 1964), consisted of placing the animal in a metabolism chamber immediately after injection with 150 µc of sodium acetate-1-C^{14} per kilogram body weight. The chamber was swept with CO_2-free air and expired $C^{14}O_2$ was trapped in 0.1 N sodium hydroxide. The contents of the collection trap was changed each ten minutes the first hour after injection, each 20 minutes the second hour and each 30 minutes for the remainder of the eight hour period. Barium chloride was added to a portion of the contents of the sodium hydroxide trap and the resulting barium carbonate was dried, weighed and sonicated in dioxane-ethanol counting solution for radioactivity assay. Total CO_2 output was determined by titrating another portion of the carbonate trapping solution. Three experimental and three control animals were used.

The results confirmed our previous observation that the rate of conversion of radioactive acetate to carbon dioxide is reduced in the experimental animal as shown by the specific activity of expired CO_2 (Figure 6). The half-life, as calculated from the data in Figure 6, is 44 minutes for the control animals and 68 minutes for the experimental animals. Titration data show no difference between the control and experimental animals in total CO_2 output. The slower turnover rate is also reflected by a calculation of the percent of injected isotope recovered in carbon dioxide at various periods after injection. For example, only 12.53% of the injected isotope was found in carbon dioxide after twenty minutes in experimental animals compared to 17.04% in control animals. By four hours after injection, the values for experimental and control animals were 48.74% and 46.69% respectively. The difference in rate of recovery of isotope is dramatically demonstrated in Figure 7 where the percentage of injected isotope recovered in CO_2 from experimental animals is plotted as a percent of control values at various times after injection.

Interpretation of these data is less subject to speculation than previously because of the availability of several pieces of

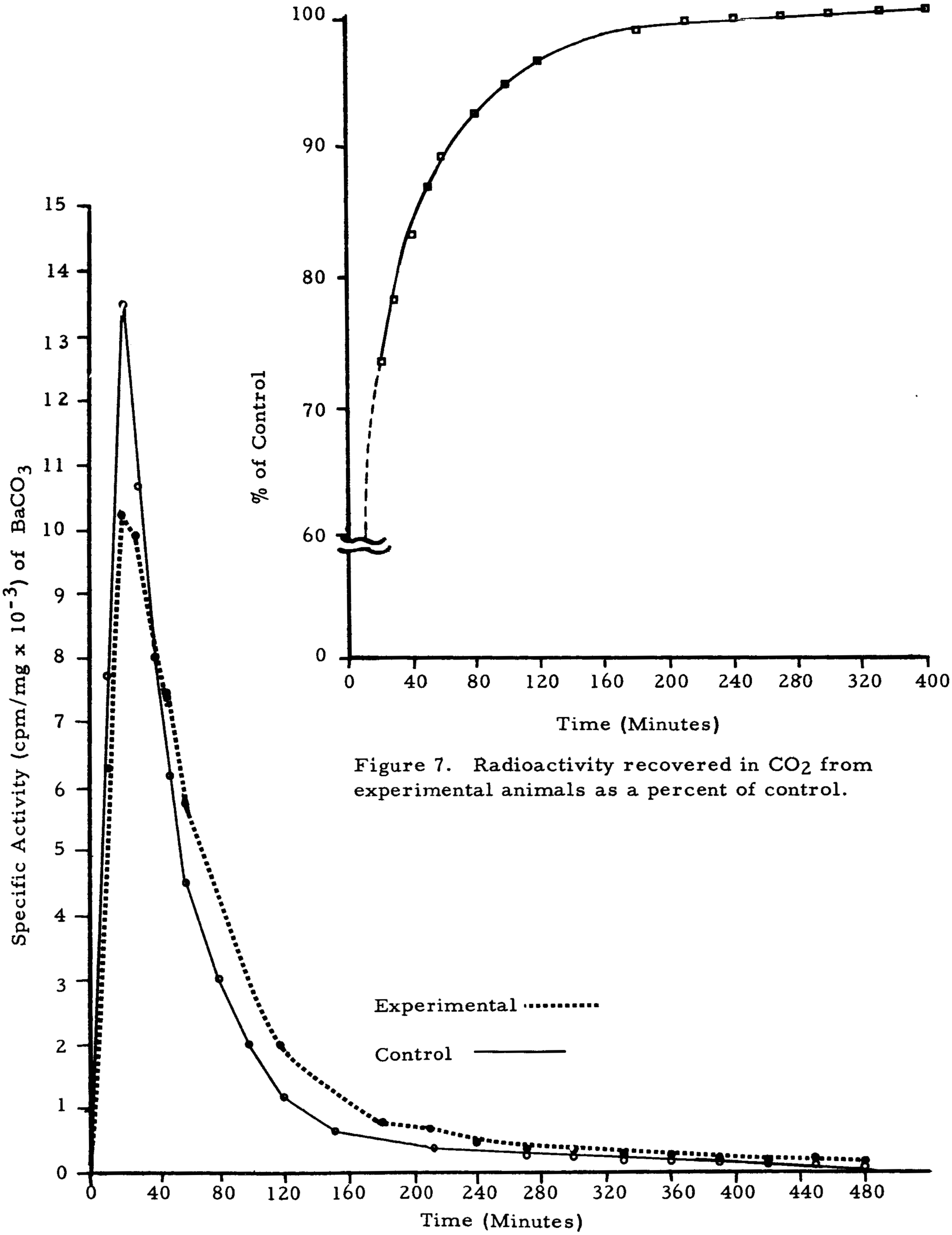

Figure 7. Radioactivity recovered in CO_2 from experimental animals as a percent of control.

Figure 6. Expiration of Radioactive CO_2 During First Eight Hours after Injection of C^{14}-Acetate. 4-Week Exposed Animals.

evidence. For example, the data obtained relative to $C^{14}O_2$ output from acetate-1-C^{14} could reflect a difference in the mechanism for CO_2 expiration rather than a difference in acetate utilization. Preliminary evidence, using three experimental and three control animals, indicates that injected sodium bicarbonate-C^{14} is expired at the same rate in experimental and control animals, showing that the observed differences are, in fact, associated with acetate utilization. Further data on this point will be collected from the experiment now in progress.

Qur concern has previously been expressed that a difference in the size of the acetate pool in experimental and control animals could account for the decrease in the rate of utilization of injected acetate. Careful examination of specific activity data (Figure 6) shows that such an interpretation is not valid. Although the lower specific activity of respiratory CO_2 from experimental animals up to forty minutes post injection could be accounted for by a larger acetate pool in these animals, the higher specific activity after forty minutes could not. In addition, direct measurement of the acetate pool in the liver (see Section IV) indicates no difference between experimental and control animals.

Finally, two pieces of evidence indicate that the observed differences in acetate utilization are a result of long term exposure to the test environment rather than metabolic changes associated with removal of experimental animals from the test environment for study at atmospheric pressure. First, the fact that incorporation of acetate into liver lipids of experimental animals was the same whether the animal was maintained in the test environment or allowed to metabolize at atmospheric pressure (see Section VI) indicates that this measure of acetate utilization was not affected by short term environmental changes. Second, the turnover rate for acetate-1-C^{14} to CO_2 in animals exposed to the test environment for 12 weeks did not differ from controls, even though the 12 week animals were removed from the test environment and returned to atmospheric pressure for study.

Thus, we conclude that the ability of the experimental animals to utilize the injected acetate is impaired as a result of exposure to the high oxygen, low pressure environment. The radioactivity data show a delay in the utilization of injected acetate although production of carbon dioxide from endogenous substrates continues, since no difference in total CO_2 output was observed. This delay in utilization of injected acetate is probably associated with the decrease in coenzyme A levels in experimental animals as previously reported.

VIII. DISCUSSION

The data presented over the last three status reports all point to adaptation of rats to the high oxygen-low pressure environment with prolonged exposure. For many of the parameters, animals were exposed continuously for 12 weeks before measurements returned to levels found in the control animals.

With respect to turnover rates of liver, heart and kidney lipids, greater differences were observed in the control animals as a function of the age of the animals than between the experimental and control animals after 12 weeks of exposure (see October, 1965 Status Report). Heart was the only tissue whose weight was significantly greater in the experimental animals, and this occurred only after 12 weeks of exposure.

Blood gas analyses suggest that the tissues were exposed to a hyperoxic state at 4 weeks, nearly normal with respect to pO_2 after 8 weeks, and were essentially hypoxic by the time the animals have been exposed to the test environment for 12 weeks (see April, 1965 Status Report). Thus, there appeared to be some overcompensation.

The C^{14}-turnover rate data were based upon the assumption that the size of the acetate pool was not appreciably different in the two groups of animals. Experimental evidence presented in this report tends to support this assumption. It is also interesting that the amount of CO_2 expired by the experimental animals was the same as that observed in the control animals. Thus, it is not too surprising that the experimental animals are no smaller than the comparable control animals after 4, 8 or 12 weeks of exposure (see April, 1965 Status Report).

Consequently it is somewhat difficult to explain and interpret the delay observed in the $C^{14}O_2$ specific activity curve of the experimental animals injected with acetate-1-C^{14} after 4 weeks of exposure. Also, it appeared that the turnover rate (based on half-life) was somewhat slower in the experimental animals after 4 weeks of exposure (slower by about a factor of 46%) but not after 12 weeks. These data compare very closely with the coenzyme A concentration in brain (see October, 1965 Status Report). Perhaps the most plausible explanation assumes that neither the acetate nor the bicarbonate pool is altered by exposure of the animal to the high oxygen-low pressure environment. Since the experimental animals were no smaller than the comparable control animals and no difference in food or water consumption was observable (see April, 1965 Status Report), no gross decrease in metabolic rate is anticipated. Perhaps in the 4 week exposed animals, coenzyme A concentration was limiting thus limiting the amount of acetyl-CoA entering the TCA cycle from acetate or fatty acid oxidation. If such occurred, the remainder of the CO_2 expired would be derived from other endogenous sources (particularly the oxidation of glucose-6-phosphate via the pentose phosphate pathway). In other words, the relative contribution of acetyl-CoA and other endogenous sources of CO_2 may be altered. This hypothesis is consistent with the fact that the accumulative expiration of $C^{14}O_2$ from acetate-1-C^{14} is the same in the two groups of animals only after 4 hours following injection of the acetate (see Section VII in this report). It is impossible to explain these data by proposing a difference in either acetate or bicarbonate pool size.

It is extremely interesting that no significant change in adrenal function could be detected but a depletion of adrenal ascorbic acid was observed after 2 and 4 weeks of exposure.

The ascorbate data are consistent with the data obtained for other easily oxidizable substances found *in vivo* (see April, 1965 and October, 1965 Status Reports). The adrenal function data suggest that adaptation to the high oxygen-low pressure environment is not too stressful on the animal. However, two pieces of evidence give some cause for alarm with respect to astronaut fitness and function during prolonged flights in this environment: (a) The increased heart weight after 12 weeks of exposure, and (b) The 33% decrease in eye lipids after only 4 weeks of exposure. One would not particularly anticipate adaptation in regard to the eye lipids since these tissues are continuously exposed directly to the hyperoxic state of the animal chamber while internal tissues and organs depend solely on the transport of gas by the blood as the oxygen source.

IX. APPENDIX

The following is a preprint of the abstract of a paper entitled "Metabolic Adaptation of Rats to a High Oxygen-Low Pressure Environment" by John Patrick Jordan, J. B. Allred and A. D. Bond which will be presented at the Fall Meeting of The American Physiological Society in Houston, Texas, 29 August-2 September, 1966.

METABOLIC ADAPTATION OF RATS TO A HIGH OXYGEN-LOW PRESSURE ENVIRONMENT

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The effects of prolonged exposure to a space cabin environment (O_2 at 5.2 psia) on the metabolism of rats were studied. Control animals were housed in an identical chamber in which the gas phase was air at 1 atm. Throughout 12 weeks of continuous exposure to the test environment, body and tissue weights did not differ significantly between the two groups of animals except for a greater heart weight in the experimental animals. However, using tissue composition and C^{14}-turnover rate data obtained from injection of acetate-1-C^{14} at varying times prior to sacrifice, several parameters suggest adaptation. The rate of $C^{14}O_2$ expiration, coenzyme A concentration in brain, liver and kidney as well as hematocrit values and hemoglobin concentration decreased after 4 weeks of exposure but essentially returned to control values after 12 weeks of exposure. Tissue cholesterol concentrations were increased in the 4 week exposed animals and tended to return to normal values after 12 weeks of exposure. C^{14}-turnover rate data showed greater differences between the two groups of animals after 4 weeks than after 12 weeks. However, not all metabolic parameters returned to control values, e.g. C^{14}-turnover rates of liver lipid and tissue carbohydrate were greater in experimental rats after 12 weeks but the turnover rate of heart cholesterol was less. Steady state analyses of tissues and carcass indicate an increase in carbohydrate and a decrease in lipid, particularly the saponifiable fraction, with prolonged exposure. We conclude that adaptation to the environment involves a change in the importance of certain metabolic pathways.

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